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PLANTS

Derivation of Fluorometric Chlorophyll and Pheophytin Equations

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Equations for calculation of chlorophyll *a* and pheophytin *a* by fluorometry have been cited in a number of publications. None of them, however, includes the derivation of the equations to substantiate their validity. The derivation is given here to let the user apply the equations with confidence.

Scientific literature contains quite a number of publications dealing with the determination of chlorophyll *a* in environmental samples by using fluorometry and improvements of the standard method. None of the publications, however, includes the derivation of the 2 equations used in the calculation process: one for the concentration of chlorophyll *a* and the other for the concentration of pheophytin *a* (1–3). *Standard Methods for the Examination of Water and Wastewater* (3) gives these equations as

$$\text{Chlorophyll } a, \text{ mg/cu. m} = Fs \frac{r}{r-1}(Rb - Ra)$$

$$\text{Pheophytin } a, \text{ mg/cu. m} = Fs \frac{r}{r-1}(rRa - Rb)$$

where Fs = calibration factor of sensitivity setting S to convert fluorometric readings to concentrations of chlorophyll *a*; Rb = fluorescence of extract before acidification; Ra = fluorescence of extract after acidification; r = ratio of Rb/Ra.

Derivation

The following derivation of the equations (using some different symbols), which substantiates the validity of the published equations, should be of interest to analytical chemists who use the chlorophyll *a* method.

Definition of symbols:

F = fluorescence of sample
R = fluorescence of pure chlorophyll *a* standard
Fb and Rb = total fluorescence before acidification
Fa and Ra = total fluorescence after acidification
Fc and Rc = fluorescence of chlorophyll *a*
Fp and Rp = fluorescence of pheophytin *a*
Fcp and Rcp = fluorescence of chlorophyll *a* converted to pheophytin *a* by acidification
S = calibration factor for appropriate sensitivity setting

A pure chlorophyll *a* standard contains only chlorophyll *a* and no pheophytin *a*, but a chlorophyll *a* sample may contain pheophytin *a*. The ratio of an unacidified pure chlorophyll *a* standard to an acidified pure chlorophyll *a* standard in fluorescence can be considered constant for each analytical setup (1, 3, 4) and is represented as:

$$\frac{Rb}{Ra} = r$$

The fluorescence of a sample before acidification is described by:

$$Fb = Fc + Fp \quad (1)$$

Solving for Fc,

$$Fc = Fb - Fp \quad (2)$$

The fluorescence of a sample after acidification is described by:

$$Fa = Fp + Fcp \quad (3)$$

Solving for Fcp,

$$Fcp = Fa - Fp \quad (4)$$

If $\frac{Rb}{Ra} = r$, then $\frac{Fc}{Fcp} = r$, and

$$\frac{Rb}{Ra} = \frac{Fc}{Fcp} = \frac{Fb - Fp}{Fa - Fp} \quad (5)$$

Solving Equation 5 for Fp yields

$$Fp = \frac{RaFb - RbFa}{Ra - Rb} \quad (6)$$

which is the fluorescence of pheophytin *a* in the sample. The concentration of pheophytin *a* in the sample is obtained by using a pure chlorophyll *a* standard. When this standard is acidified, all chlorophyll *a* is converted to pheophytin *a*, whose fluorescence is only 42% that of chlo-

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rophyll *a* (1). When, therefore, the concentration of pheophytin *a* is calculated using a pure chlorophyll *a* standard, the fluorescence of pheophytin *a* must be multiplied by *Rb*/*Ra*. Equation 6 then becomes:

$$Fp = \frac{Rb}{Ra}\left(\frac{RaFb - RbFa}{Ra - Rb}\right), \quad \frac{Fb}{Fa} \leq \frac{Rb}{Ra} \qquad (7)$$

Multiply numerator and denominator by −1:

$$Fp = \frac{Rb}{Ra}\left(\frac{RbFa - RaFb}{Rb - Ra}\right)$$

$$= \frac{Rb}{Rb - Ra}\left(\frac{RbFa - RaFb}{Ra}\right)$$

Divide numerator and denominator by *Ra*:

$$Fp = \frac{\frac{Rb}{Ra}}{\frac{Rb - Ra}{Ra}}\left(\frac{Rb}{Ra}Fa - Fb\right)$$

$$= \frac{\frac{Rb}{Ra}}{\frac{Rb}{Ra} - 1}\left(\frac{Rb}{Ra}Fa - Fb\right)$$

Substitute *r* for *Rb*/*Ra*

$$Fp = \frac{r}{r - 1}(rFa - Fb)$$

The concentration of pheophytin *a* is then described by:

$$\text{Pheophytin } a = \frac{r}{r - 1}(rFa - Fb)S \qquad (8)$$

Substituting Equation 6 into Equation 2 yields

$$Fc = Fb - \left(\frac{RaFb - RbFa}{Ra - Rb}\right) \qquad (9)$$

$$= \frac{RaFb - RbFb - RaFb + RbFa}{Ra - Rb}$$

$$= \frac{-RbFb + RbFa}{Ra - Rb}$$

Multiply numerator and denominator by −1:

$$Fc = \frac{RbFb - RbFa}{Rb - Ra}$$

$$= \frac{Rb}{Rb - Ra}(Fb - Fa)$$

Divide numerator and denominator by *Ra*:

$$Fc = \frac{\frac{Rb}{Ra}}{\frac{Rb}{Ra} - 1}(Fb - Fa)$$

Substitute *r* for *Rb*/*Ra*:

$$= \frac{r}{r - 1}(Fb - Fa)$$

The concentration of chlorophyll *a* is then described by:

$$\text{Chlorophyll } a = \frac{r}{r - 1}(Fb - Fa)S \qquad (10)$$

The above derivation arrived at the same equations that are being recommended in a number of publications. Analytical chemists can, therefore, use the equations with confidence.

The above derivation shows the validity of the published equations only. Interferences by other chlorophylls and derivatives should be considered separately.

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